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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF EXTRACTS FROM THE ROOT BARK OF *CARISSA EDULIS*, AGAINST HUMAN / ANIMAL PATHOGENS

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ABSTRACT
The aim of this paper was to confirm the folkloric claim that *C. edulis* treats tuberculosis. To validate this tribal claim, the phytochemicals screening was determined. The root barks were tested against some human ailments. Extracts from the root-bark of *Carissa edulis* were macerated with different solvents. All fractions were subjected to phytochemical screening and antimicrobial activity using the disc-diffusion method. The extracts contained alkaloids, sterols and resin. The ethanol and the pet-ether fractions were active on *Staphylococcus aureus* and *Escherichia coli* respectively, at high concentrations. This attests that *Carissa edulis* contains bioactive compounds of potential therapeutic and prophylactic significance and supports the claim for its treatment of bacterial and fungal infections. Further analyses could pave way for candidature as phyto-therapeutic agents against some bacterial infections.

KEYWORDS: *Carissa edulis*, root-bark, phyto-therapeutic, *Staphylococcus aureus*, gram-positive, *Escherichia coli*

INTRODUCTION

Plants serve as the basis of traditional medicine systems for thousands of years in Nigeria, India, Chin, Indonesia etc. (Hammer, 1999). In Kano, Nigeria, tuberculosis is a health challenge (FHI, 2001; Nwankwo *et al.*, 2005; Emokpae *et al.*, 2006) and is believed that *Carissa edulis, vahl (Apocynaceae*) possesses medicinal properties effective in the management of tuberculosis and other ailments. It is used traditionally for the treatment of headache, chest complaints, rheumatism, gonorrhea, syphilis, rabies and as a diuretic and cancer (Nedi *et al*., 2004). The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw materials for addressing medical disorders and pharmaceutical requirements. Collectively, plants produce diverse arrays low molecular mass natural products also known as secondary metabolites, (Fatope, 2001).

Most developing countries have adopted traditional medical practice as an integral part of their culture. Some plants contain high calcium in their cell walls which has made them suitable for the growth of mineral crystals. The presence of phosphorus in them can be exploited for synthesizing hydroxapatite, thus, utilizing the traditional bone-fracture healing in advanced techniques of new material synthesis. (Habibovic, *et al*., 2002) This knowledge has led to the interest by pharmaceutical companies as a resource for research and development programmes in the pursuit of discovering novel drugs.

The medicinal value of these metabolites is due to the presence of chemical substances that produce definite physiological action on the human body. Some of the valuable ones include: alkaloids, glucosides, Steroids, flavonoids, terpenoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, body building (Chidambara *et al*., 2003). There are reports of antibiotic resistance of human pathogens, to available antibiotics (Mitsuyama *et al*., 1987; Gutmann *et al*., 1988; Mathias *et al*.,., 2000; Ganguly, *et al*., 2001; Martino *et al;* 2002). Biomolecules of plant origin appear as alternatives for the control of these and some human pathogens and their uses have been shown to have scientific basis, since chemical compounds found in the various species have medicinal effects.

Carissa edulis (Hausa, *Chizake* or *lemun-daji*) is a crottalabe overlapping to the right, anthers not tailed, ovary syncarpus; fleshy, branches usually armed with axillary spines. Spiny shrup up to 5m high. Its leaves

ovale usually acute at apex, shortly cuneate or rounded at base, 2-6cm long, and 2-5cm broad, with 3-5 pairs of lateral nerves, glabrous or pubescent. Petiole short (Festus *et al*; 2006).

C. edulis has been used in Nigeria in forms of decoction and concoctions for the cure of cough, catarrh, diarrhea and tuberculosis (Aja afar,1982.). This paper reports the potentials of *C. edulis* for its anti-microbial activity.

MATERIALS AND METHODS

Sampling

Fresh samples of the root- bark of *Carissa edulis* was collected in June, 2006 from a local medical practitioner at Zerewa town Rogo local Government area of Kano State Nigeria. It was identified and authenticated according to Hutchinson and Dalziel (1956). The sample was air dried in the laboratory before being powdered using pestle and mortar to a mesh size of about 60 and then stored in a dry container.

Extraction

Eight hundreds grams (800g) of the powdered roots of the plant was percolated with (5Lt) of distilled ethanol for two-weeks. After which it was decanted, filtered, and concentrated on rotary evaporator (R110) at 40^0C to obtain ethanol soluble fraction, (F_E01), weight, 17.73g. (Crude).

F_{E01} was macerated with petroleum ether and concentrated with (R110) at 40^0C, to obtain pet ether soluble fraction. It weighed 6.30g and was labeled ($F_{PE}01$). Successive maceration and concentration with (R110) at 40^0C processes continued with different solvents thus, ethyl acetate, 2.9g ($F_{EA}01$), Chloroform 5.1g(F_C01), and acetone 2.2g (F_A01), with an insoluble junk.

Anti-microbial testing

The disc`s were prepared using a What-man filter paper by punching and were kept in vial-bottles and sterilized in an oven at 150^0C for 15minutes.

Preparation of stock solution.

Five different extracts (F_E01), ($F_{PE}01$), ($F_{EA}01$), (F_C01), and (F_A01) were used for both analysis. Concentrations (in triplicates) were produced from each extract at 5000, 2500, 1000, and 500µg / (cm^3) respectively using the dilution formula (Almagboul, using pestle and mortar to a mesh size of about 60 and then stored in a dry container.

Preparation of culture medium

Four bacteria species: *Staphylococcus aureus, Escherichia coli, Klibsiela. pneumonia and Streptococcus species* stock cultures were collected at Murtala Mohammed Specialist Hospital Kano; These organisms were identified in the Microbiology Department of Bayero University Kano.

Sensitivity test

Approximately eleven grams (11.24g) of the nutrient agar were dissolved in 400cm^3 of distilled water, shaken and sterilized in an autoclave in the microbiology laboratory for 15 minutes at 121^0C. 20 cm^3 of the mixture each was then pippetted into Petri-dishes with the help of heat in order to kill any species around and avoid contamination. The samples plates were allowed to set and later incubated aerobically at37^0C then turn upside down in the ovum for them to dry.

Inoculation of the test organisms on nutrient agar-prepared plates were prepared as reported by Fatope *et al* ;(1993) was achieved by; flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. Prepared disks containing the fractions at different concentrations were placed on the inoculated plates using sterilized forceps in each case (Fatope *et al*; 1993); the plates were then turned upside-down and incubated at 37^0C for 18 h.

Scoring and Reading

The result was taken by considering the zone of growth and inhibition of the organism by the various concentrations of the test fractions (Mackie *et al*; 1989).Activity and inactivity were observed in accordance with the standard and acceptable method and results tabulated as shown in Table I.

Phytochemical screening

All soluble fractions were subjected to phytochemical screening in accordance with the standard procedure (Harborne 1973and 1992), Alkaloids, steroids, carbohydrates tannins, saponnins and resin tests were carried out on all the fractions. The results obtained are shown in Tables II.

RESULTS AND DISCUSSION

Antimicrobial efficacy of different solvent extracts of root-bark of *Carissa edulis* against human pathogen bacteria (Zones of inhibition in millimeters).

Table I. Antimicrobial activity of the extracts from root-bark of:

Plant	Fraction	Microorganism			
		E. coli	*K.pneumonia*	*S. aureus*	*S.species*
Carissa edulis	Ethanol	13.50±0.10	8.32±0.16	-	-
	Pet- ether	19.45±0.14	17.36±0.13	21.15±0.16	6.54±0.10
	Ethyl acetate	09.05±0.44	06.32±0.43	05.32±0.43	-
	Chloroform	14.51±0.11	15.51±0.11	09.00±0.44	-
	Acetone	02.13±0.14	05.43±0.21	05.43±0.21	-
Control	Gentamicin	21.65±0.10	13.85±0.15	14.65±0.15	9.50±0.10

Table II Phytochemical analysis of root-bark of *Carissa eduli:*

Test for:	Ethanol	Pet. Ether	Ethyl acetate	Chloroform	Acetone
Alkaloids.	+	+	+	+	+
Saponnins	-	-	-	-	-
Sterols	+	+	-	+	+
Resin	+	+	-	+	+

Key: Absent (-); Present (+)

From the results, the ethanol extract is active only on the *E. coli*, distorted on the *K. pneumonia* and inactive on other bacteria. The pet-ether extract showed the highest activity at all concentrations with zone of inhibition of 21.15 mm on the *S. aureus*. This followed by the chloroform fraction at higher concentrations (>1000) with an inhibition zone of 15.51mm, though moderately active on the other microbes. This may be due to its oxidation to phosgene. The ethyl acetate and acetone fractions are active on three of the micro-organisms. *S. species* is inactive to all the fractions. It must have developed resistance against the extracts. Other observation is that there are morphogenetic and phenotogenical variations of

plants harvested at the vegetative, floral budding, full flowering, fresh fruiting and mature fruiting stages (Cuneyt *et al*; 2007).

Phytochemical screening portrays that most of the natural products tested for were present in the plant material except saponnins. This shows the generality of the components in medicinal plants. Biological actions are due to these components in complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties. To a large extent, the phonological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction are possible sources of variation in the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982). It is difficult though, to make connections between the present findings and the acclaimed efficacy of these plants on the management of tuberculosis. However studies have indicated that certain biflavonoids have inhibitory activity against *Mycobacterium* that causes tuberculosis (Lin *et al.,* 2001) and other components that act in similar way as the Isoniazid, Rifampin and Pyrazinamide, antituberculosis drugs (Sadoff, 2006) towards patients utilizing the plant studied in this work.

CONCLUSION

These results suggest that, pet-ether and chloroform extracts of *Carissa edulis* probably contain active agent(s) and this provides the basis for the folkloric claim and could be a promissory candidate for drug development and validate the tribal claim, as a cure for tuberculosis and some human ailments. This assertion is confirmed, as their extracts indicate the presence of phytochemicals. It is suggested that more work be conducted that will further elucidate and characterize the active components and possible mechanism involved in the use of these plants in the ethno medical practices.

RECOMMENDATION

It is desirable that more effort, more research, more support, and more funding be encouraged specially in valorizing our natural patrimony as well as conducting more scientific researches on local herbs. This will ensure that the entire ethno- flora of the sanctuary be documented in a way that information about sustainable uses of plants is conserved. Our base will thus be strengthen and it will foster greater compatibility between orthodox and ethno medical claims paving the way to the discovery of "lead" compound(s) to our present day ailments.

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